

Multiplex Measurements of Cytokines in High Density Formats Using Multi-Array™ Technology

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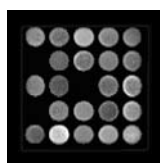
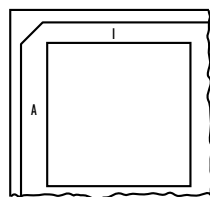
1 Abstract

We present multiplexed immunoassays for human cytokines using Meso-Scale Discovery's (MSD's) Multi-Array™ platform. These assays were conducted in MSD's new Multi-Spot™ plates, using a sample kit provided by MSD. The kit includes plates pre-coated with capture antibodies for each cytokine, a cocktail of labeled detection antibodies and other related assay reagents.

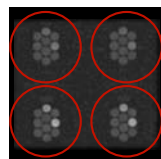
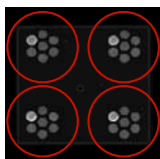
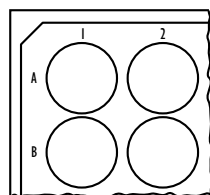
MSD's multiplex cytokine assays have no-wash formats, rapid protocols (2.5 hours) and fast read times (1 minute per plate). They are compatible with complex assay matrices (e.g. cell supernatants, cell lysates, serum, blood). Assays require as little as 10 µl of sample, and have excellent selectivity (99.5%) and sensitivity (~ 1-10 pg/ml). The wide dynamic range (>3 logs) allows users to avoid multiple dilutions.

We provide comprehensive performance data for a new Multi-Spot 384-well kit for measuring hIL-1β, hIL-2, hIL-6 and hTNF-α. In addition, we show data for the simultaneous measurement of 24 different human cytokines in a Multi-Spot 24-well plate format.

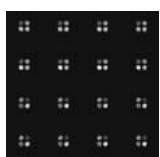
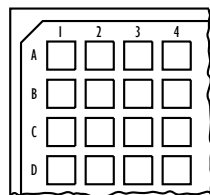
2 Multi-Spot Plate Formats



Multi-Spot 24-well plates
with 25 spots per well



Multi-Spot 96-well plates
with 4, 7 or 10 spots
per well



Multi-Spot 384-well plates
with 4 spots per well



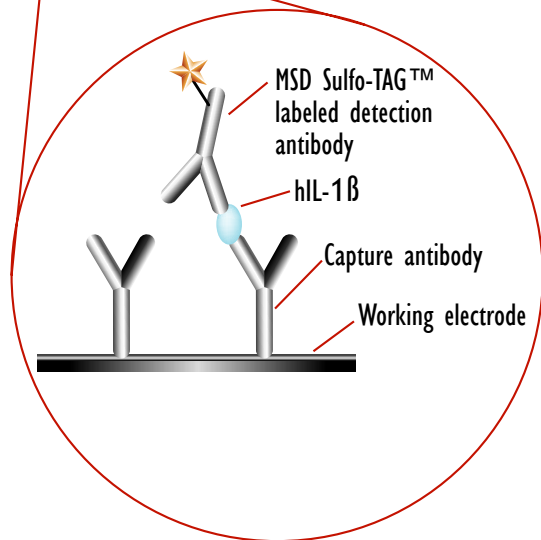
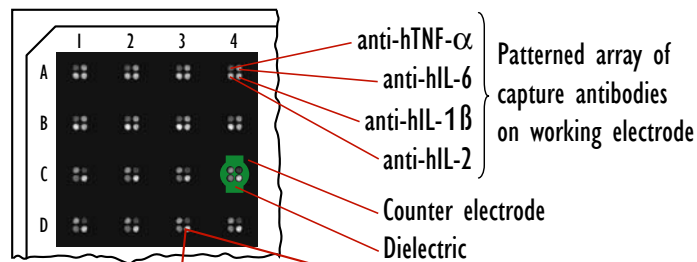
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3 Detection Kits for Multiplexed Cytokine Immunoassays

Kit Components:

- Pre-coated Multi-Spot plate: contains patterned array of anti-cytokine capture antibodies
- Detection Antibody: Labeled detection antibodies in MSD Antibody Diluent
- MSD Read Buffer: buffer optimized for ECL measurement
- Set of Standard Calibrators



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4 Assay Protocol for Multi-Spot 384-well Plates

No-Wash Format:

Add 10 μ l Sample, shake 1-2 hours at room temperature

Add 10 μ l Detection Antibody Cocktail, shake 1-2 hours at room temperature

Add 20 μ l MSD Read Buffer P (2x)

Analyze plate using SECTOR™ Imager 6000

1-Wash Format:

Add 10 μ l Sample, shake 1-2 hours at room temperature

Add 10 μ l Detection Antibody Cocktail, shake 1-2 hours at room temperature

Wash 3x with PBS

Add 40 μ l MSD Read Buffer T (1x)

Analyze plate using SECTOR Imager 6000

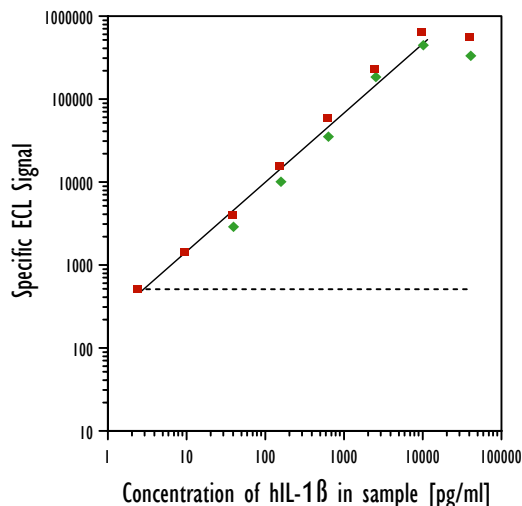
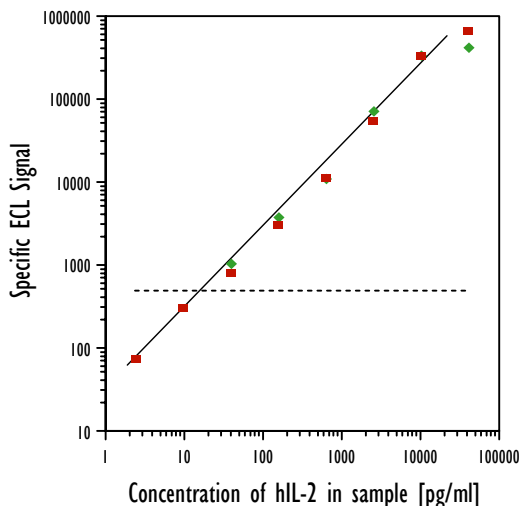
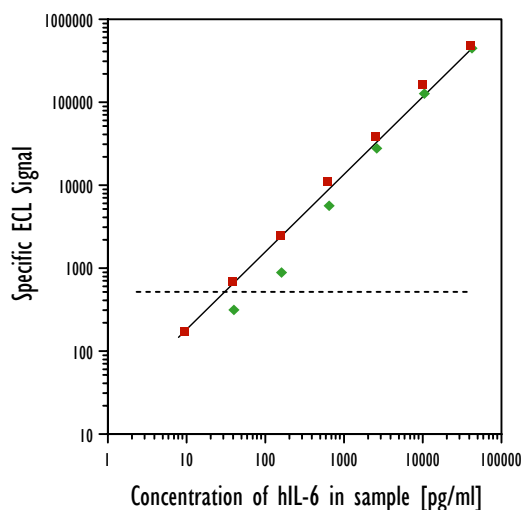
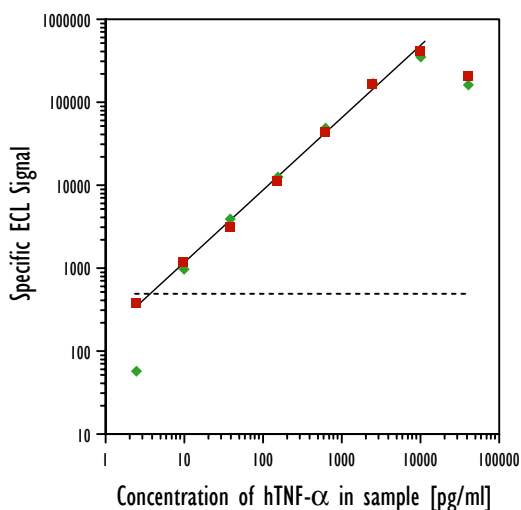
Multi-Spot 384-well Plate with 4 spots per well



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5 Performance of a Multi-Spot 384-well Assay for hIL-1 β , hIL-2, hIL-6 and hTNF- α (No-Wash Format)



Sensitivity:

~ 10 pg/ml

Dynamic Range:

10 pg/ml – 10000 pg/ml

Selectivity:

Same signal response for single and mixed calibrators.

Variability:

Average CVs <10%.

Calibrators are prepared in RPMI media containing

10% fetal calf serum

Green symbols: single calibrator

Red symbols: mixed calibrators

Dashed lines: detection limit

(25% above background signal)



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6 Sensitivity of Multi-Spot 384-well Assays for hIL-1β, hIL-2, hIL-6 and hTNF-α

Detection limits are defined by a specific signal of 25% above background. A single wash reduces the detection limit by approximately one order of magnitude.

Similar assay performance and detection limits can be achieved in Multi-Spot 96-well plates with 4, 7 or 10 spots per well.

	No-Wash [pg/ml]	1-Wash [pg/ml]
hIL-1β	6.3	0.33
hIL-2	23	2.3
hIL-6	83	13
hTNF-α	3.9	0.69

7 Cross Reactivity of a Multi-Spot 384-well Assay for hIL-1β, hIL-2, hIL-6 and hTNF-α

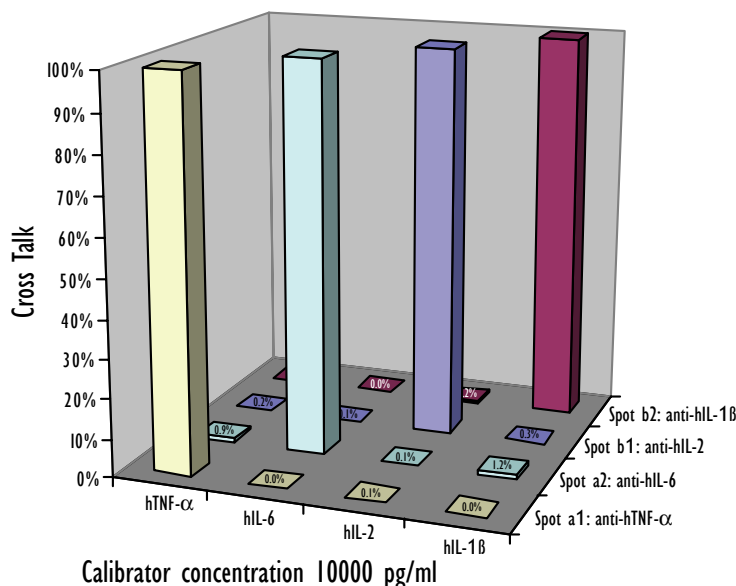
'Cross Talk' is the ratio of non-specific to specific ECL signal:

$$\frac{(\Delta\text{ECL})_{\text{non-specific}}}{(\Delta\text{ECL})_{\text{specific}}}$$

Average Cross Talk is:

0.3% (No-Wash)

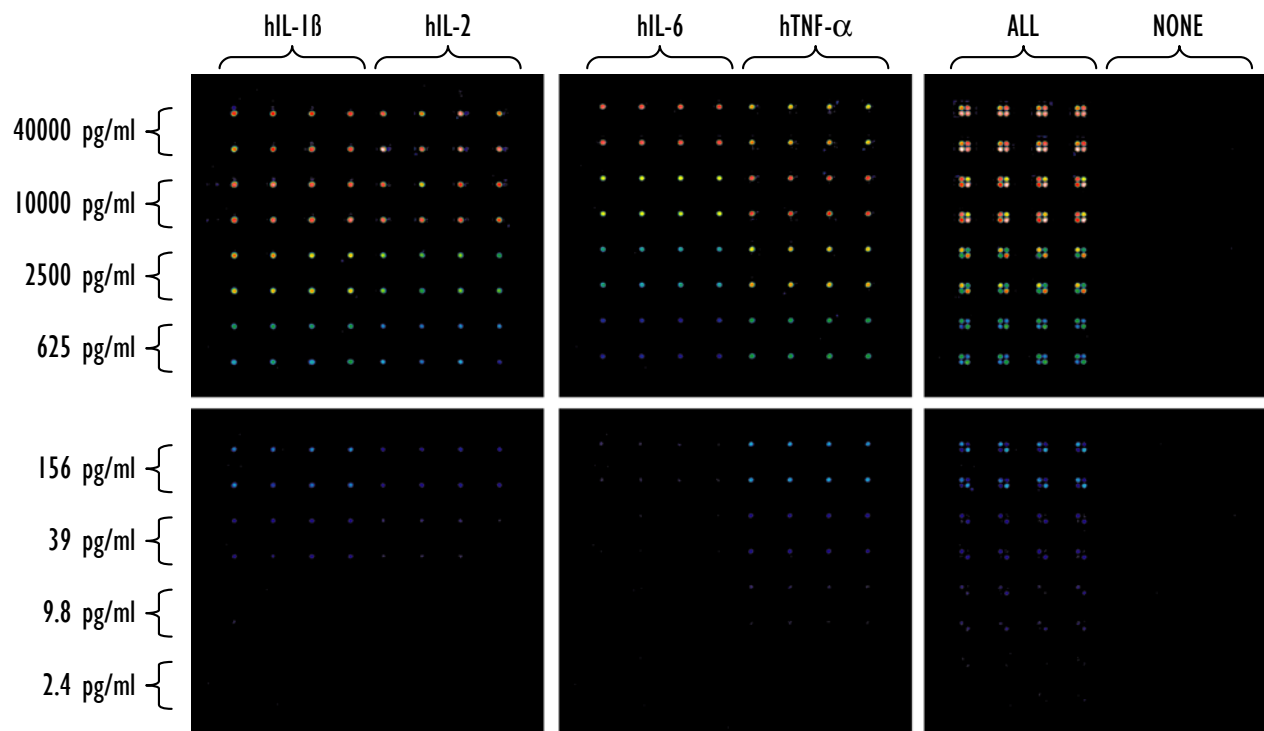
0.6% (1-Wash)



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8 ECL-Images of a Multi-Spot 384-well Plate for hIL-1 β , hIL-2, hIL-6 and hTNF- α (No-Wash Format)



average CVs <10%

9 Assay Protocol for Multi-Spot 24-well Plates

1-Wash Format:

Add 200 μ l Sample, shake 2 hours at room temperature

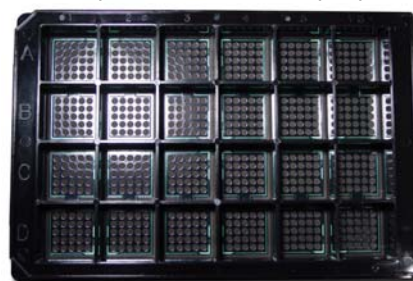
Add 200 μ l Detection Antibody Cocktail, shake 2 hours at room temperature

Wash 3x with PBS

Add 1.6 ml MSD Read Buffer T (1x)

Analyze plate using SECTOR Imager 6000

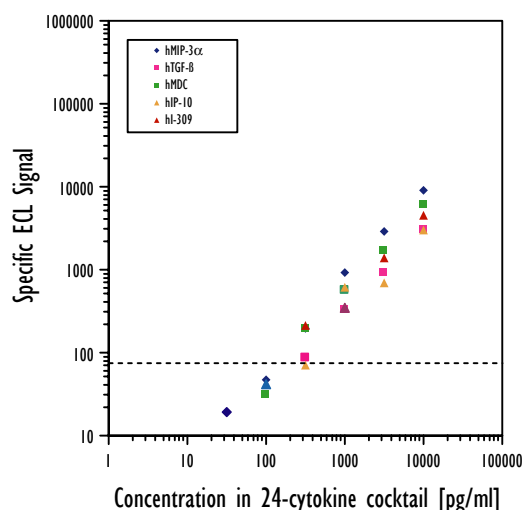
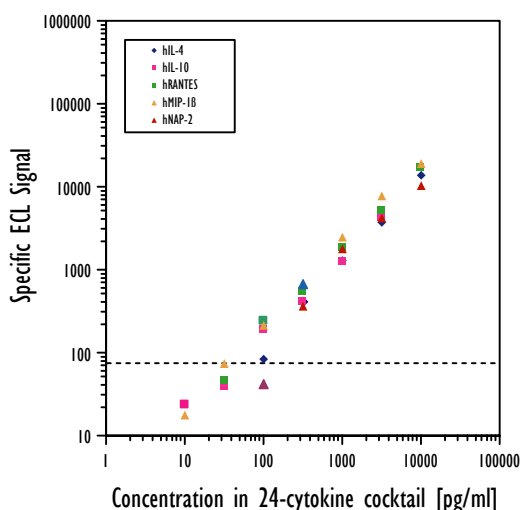
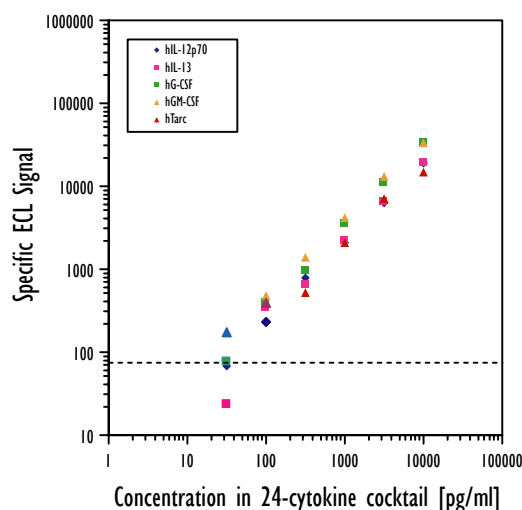
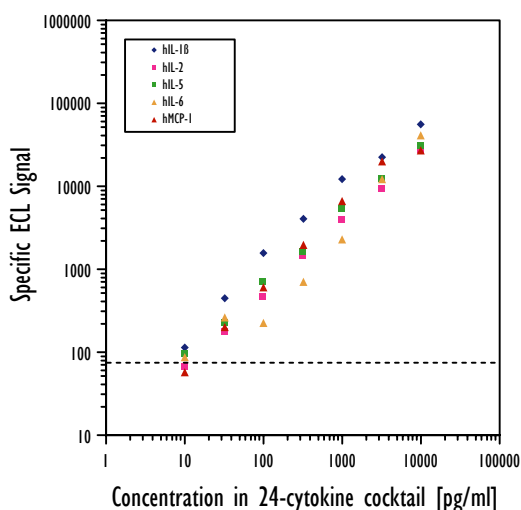
Multi-Spot 24-well Plate with 25 spots per well



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10 Performance of a Multi-Spot 24-well Assay for 24 Human Cytokines (1-Wash Format)



Sensitivity:

As low as 5 pg/ml (hIL-1B) dependent on antibody pair

Dynamic Range:

From 10 - 100 pg/ml to >10000 pg/ml

Variability:

Average CVs <10%

Calibrators are prepared in RPMI media containing

10% fetal calf serum

Dashed lines: detection limit

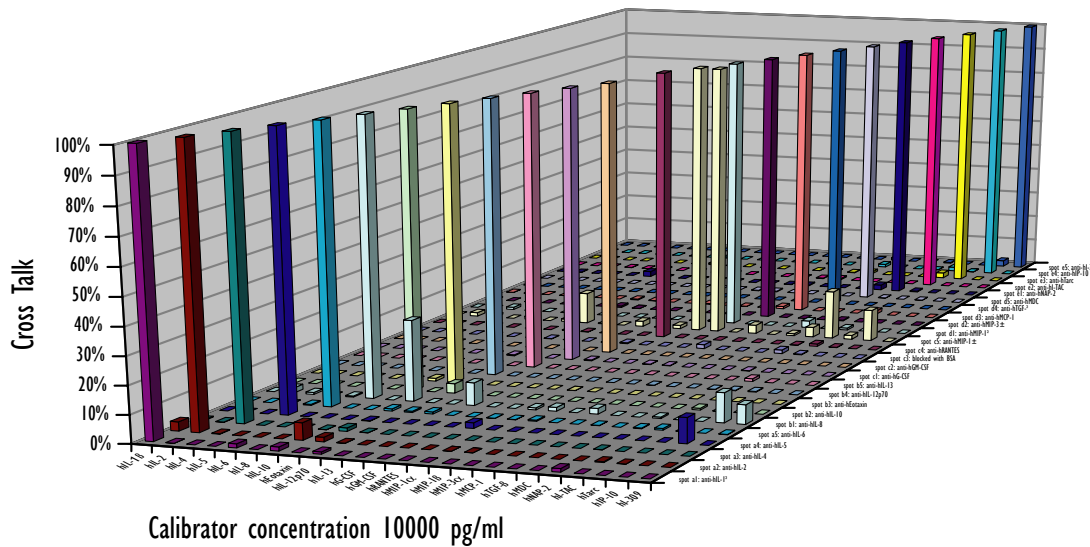
(25% above background signal)



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II Cross Reactivity of a Multi-Spot 24-well Assay for 24 Human Cytokines (1-Wash Format)



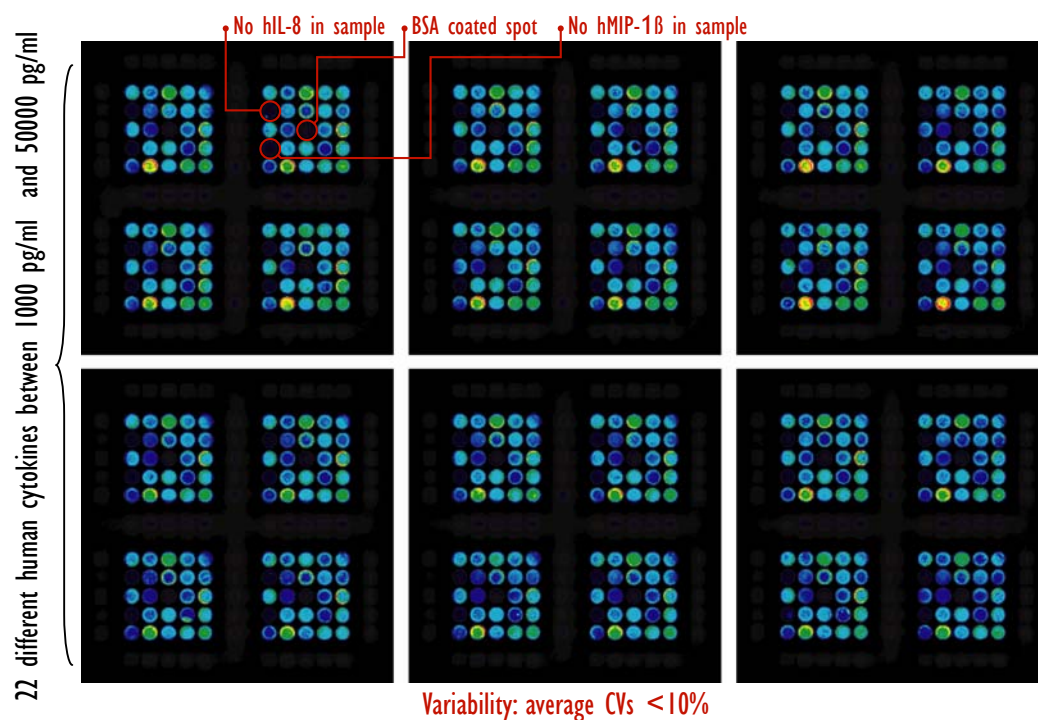
Average Cross Talk is low (<1%) – with the exception of certain homologous cytokines (e.g., hMIP-1 α and hMIP-1 β). We continue the optimization of the antibody pairs and the assay conditions to eliminate these effects.



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12 ECL-Images of a Multi-Spot 24-well Plate for 24 Human Cytokines (1-Wash Format)



13 Conclusion

Meso-Scale Discovery's new high density, multiplexed cytokine immunoassays are based on MSD's Multi-Array platform.

Multi-Spot plates are now available in 24, 96 and 384-wells formats for simultaneous measurements of 4, 7, 10, 25 and more cytokines in a single sample.

MSD's multiplexed cytokine assays have rapid protocols (2.5 hours) and fast read times (1 minute per plate); excellent selectivity (99.5%) and sensitivity (~ 1-10 pg/ml); wide dynamic range (>3 logs); small sample volumes (as little as 10 µl); no-wash and 1-wash formats.

MSD provides complete cytokine detection kits based on the new Multi-Spot plates. Plates can be custom-coated for specific applications, based on an expanding library of currently more than 40 human, mouse and rat cytokine immunoassays.



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